

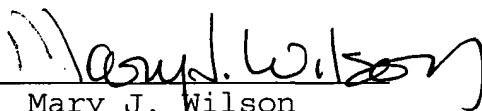
Sequence Listing does not raise the issue of new matter as the sequence information contained therein is presented in the application as originally filed. The computer readable copy of the Sequence Listing submitted herewith is the same as the attached paper copy of that Listing.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached pages are captioned "Version With Markings To Show Changes Made."

This application is submitted to be in condition for allowance and a Notice to that effect is requested.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

The paragraphs beginning at page 4, line 16:

Figure 1. Time course measurement of the outflow facility for GRGDTP (SEQ ID NO:1) ( $200\mu\text{M}$ ) perfusion in porcine cadaver eyes. Results are expressed as percent change in outflow facility and the contralateral eyes were used as sham-manipulated paired controls. Values were expressed from seven pair of samples and expressed as mean S.E.

Figure 2. Effect of RGD peptide on SC cell monolayers permeability *in vitro*. SC cells grown on transwells were treated with either GRGDTP (SEQ ID NO:1) peptide or parallel control GRGESP (SEQ ID NO:2) peptide ( $200\mu\text{M}$ ) for 1hr in complete medium. The diffusion of HRP added to the upper chambers of the transwells containing SC cells monolayers was analyzed. Percent change of HRP activity was interpreted as to assign the changes in monolayer permeability in response to the treatment of SC cells with the agents employed. The HRP activity obtained from

untreated SC cells was accounted as a basal value of 0% permeability. n represents sample number and values were expressed as mean S.E.

The paragraphs beginning at page 5, line 9:

Figures 3A-3C. Effect of RGD peptide on SC cell monolayers integrity *in vitro*. SC cells were seeded on fibronectin (10 $\mu$ g/ml)-coated coverslips and grown for 10 days as apparent confluent monolayer was formed. The SC cells monolayers were treated with complete medium either containing a control GRGESP (SEQ ID NO:2) and/or GRGDTP (SEQ ID NO:1) at the concentration of 200 $\mu$ M and 1.0 mM in Figs. 3A and 3B and incubated for 3 hrs at 37C in CO<sub>2</sub> incubator, respectively. The RGD peptide induced changes in cell separation or hole formation indicated with arrows whereas the control RGE peptide had no detectable effects even at 1.0 mM concentration of the peptide. Although, morphological changes were observed within an hr of treatment but maximum effects were noticed after 3hrs of incubation. Further, the reversal of induced morphological changes on SC cell monolayers by GRGDTP (SEQ ID NO:1) (1.0 mM) treated for 3hrs was followed for indicated period of time after removal of RGD peptide as shown in Fig. 3C. The

hole formation was decreased in a time dependent manner suggesting that the induced morphological effects are reversible. Original magnification X100.

The paragraph beginning at page 6, line 19:

Figures 5A-5F. Saggital sections obtained from 4 pairs of porcine eyes perfused with GRGDTP (SEQ ID NO:1) peptide (Figs. 5D-5F) and sham treated controls (Figs. 5A-5C) were analyzed to detect morphological changes in outflow pathway cells. The specimens perfused with peptide did not show signs of cellular toxicity in the endothelial lining of the aqueous plexi other than slight dispersion of discontinuous basement membranes as compared to sham controls. Bar represents magnification.

The paragraph beginning at page 7, line 3:

Figure 6. Effect of GRGDTP-peptide (SEQ ID NO:1) (500 $\mu$ g) on IOP in live Rabbits (n=3).

The paragraph beginning at page 13, line 20:

Porcine eyes were purchased from a commercial abattoir and perfused as enucleated whole eyes using standard

constant pressure perfusion technique with a Grant stainless steel corneal fitting, as described previously (Epstein et al, *Invest. Ophthalmol Vis. Sci.* 40:74-8116 (1999)). To prevent artificial deepening of the anterior chamber, iridotomies were performed. Perfusion medium was Dulbecco's phosphate-buffered salt solution (DPBS; GibcoBRL, Gaithersburg, MD) containing 5.5 mM D-glucose. Baseline outflow facility was determined after the eye had been perfused for 1 hr at 15 mm Hg at 25°C to obtain a steady state of aqueous outflow. After removal of the Grant fitting, the fluid of the anterior chamber was replaced with experimental perfusion medium containing the GRGDTP (SEQ ID NO:1) peptide (Sigma, St. Louis, MO) or control medium. The fellow eyes used in control experiments received only the medium for perfusion. The perfusion medium with or without peptide (200µM) was perfused for 5 hrs and the outflow facility measurements were calculated each hour of intervals. The effect of RGD peptide is expressed as the percentage change in outflow facility compared to baseline over 5 hrs in the experimental eye minus the percentage change in the control eye. Values are expressed as means  $\pm$  S.E. A paired two-tailed t-test analysis was performed.

The paragraph beginning at page 14, line 22:

The time course effect of RGD peptide on outflow facility is shown in Fig 1. In porcine eyes, perfusion of GRGDTP (SEQ ID NO:1) hexapeptide (200 $\mu$ M) caused a 46%  $\pm$  11% increase in outflow facility compared with a 9%  $\pm$  7% increase in control eyes (n=7; P= 0.027) after 5 hrs of treatment. In an attempt to perfuse control GRGESP (SEQ ID NO:2) peptide (inactive form), the effect on outflow facility as compared to the washout effects as observed with perfusion medium alone was not found. Moreover, the statistical significance was not taken into consideration for control GRGESP (SEQ ID NO:2) peptide perfusion in order to measure the washout effect in the same pair of eye.

The paragraph beginning at page 16, line 2:

To correlate the specific effects of the RGD peptide compared to a control RGE peptide, the standard device was used to measure the monolayer permeability barrier function on Transwells cell culture chambers (fibronectin-coated polycarbonate filters, 3 $\mu$ M pore size, Costar Corp.) as described by Lampugnani et al (*J. Cell Biol.* 112: 479-490 (1991)). The diffusion of horseradish peroxidase (HRP) through SC cell monolayer was determined with and without

RGD hexapeptide GRGDTP (SEQ ID NO:1) (Sigma, St. Louis, MO) or known control RGE hexapeptide GRGESP (SEQ ID NO:2) (GibcoBRL, Gaithersburg, MD). The upper chambers of the Transwells were seeded with SC cells in complete medium while the lower chamber was filled with culture media and maintained for 10 days with refeeding in every 2 days. Before the experiments, the treatment of SC cell monolayers with RGD peptide or control RGE peptide was initiated by replacement of the media from the upper chamber with complete medium (500 $\mu$ l) containing HRP (0.126  $\mu$ M, Sigma), whereas the lower chamber was being replaced with 600 $\mu$ l fresh medium. The medium from the lower compartment was collected after incubation for 1hr at 37°C in CO<sub>2</sub> incubator and the enzyme activity of HRP was assayed by colorimetric method as described by Lampugnani et al (*J. Cell Biol.* 112: 479-490 (1991)). Results were expressed as percent change in enzyme activity as compared to untreated controls. Values were obtained from three independent experiments and expressed as means  $\pm$  S.E. A paired t-test analysis was also performed with respect to untreated controls.

The paragraph beginning at page 18, line 24:

To demonstrate the specific effects of RGD peptide on permeability characteristics of SC cell monolayers in

vitro, the diffusion of horseradish peroxidase (HRP) as tracer was examined in presence of GRGDTP (SEQ ID NO:1) peptide (200 $\mu$ M) or control GRGESP (SEQ ID NO:2) peptide (200 $\mu$ M). The results presented in Fig. 2 reveal that GRGDTP (SEQ ID NO:1) peptide caused a significant increase in SC cells monolayer permeability, to an extent of 64%  $\pm$  12% (n=7; P=0.0014) whereas control GRGESP (SEQ ID NO:2) showed a slight increase 12%  $\pm$  3% (n=7; P=0.0025) as compared to untreated controls.

The paragraph beginning at page 29, line 7:

To gain information of the characteristics of RGD peptide effects on SC cell monolayer integrity, the RGD peptide was used at two different concentrations (200 $\mu$ M or 1.0mM). When confluent SC cells monolayers were incubated with GRGDTP (SEQ ID NO:1) peptide (200 $\mu$ M) in complete medium, the morphologically detectable effects were observed like tiny discontinuities compared to the compact cellular morphology in normal or control GRGESP (SEQ ID NO:2) peptide (200 $\mu$ M) treated monolayers as shown in Figs. 3A. These tiny discontinuities are apparently by the dissociation of adjacent cells but not by the detachment of cells. However, at higher concentration of RGD peptide (1.0 mM) treatment showed an enlargement of gaps in SC cells



monolayer and ended up with the appearance of discontinuous hole formation but no such effect was noted with the inactive analog GRGESP (SEQ ID NO:2) (1.0 mM) as shown in Fig. 3B. These results are consistent with the effects of RGD peptide on endothelial cell monolayer integrity (Lampugnani et al, *J. Cell Biol.* 112: 479-490 (1991)). In Fig. 3C, the reversibility of the hole formation was followed after removal of RGD peptide from the incubation media and incubation was followed as time indicated. The hole formation decreased progressively in a time dependent manner suggesting that the effects of the RGD peptide are reversible even at higher concentration of peptide treatment. Moreover, the effect of RGD peptide on actin filaments did not appear to be altered but irregular bundling like appearance could be due to altered orientations of the cells around the holes. Similarly, the diffused focal adhesions appeared comparable to controls presumably due to the loosening of the focal contacts with ECM as shown in Fig. 4F.

The paragraph beginning at page 20, line 22:

New Zealand White rabbits of approximately five pounds were used for this experiment. Baseline Intraocular Pressure (IOP) data was obtained using a MENTOR tonopen

prior to anaesthesia. The rabbits were anaesthetized with IM KETAMINE and topical proparacaine. The GRGDTP (SEQ ID NO:1) peptide (500 $\mu$ g) was dissolved in phosphate buffered saline (PBS) and injected into the anterior chamber of only one eye. Further intraocular pressure measurements were performed with a tonopen and data recorded in a time dependent manner, as shown in Fig. 8.

**IN THE CLAIMS:**

6. (Amended) The method according to claim 3 wherein the compound comprises the peptide GRGDTP (SEQ ID NO:1) or mimetic thereof or derivative thereof.